

Construction of JB-3120-2, a plasmid containing T7 H91A *hin47* (+ leader)

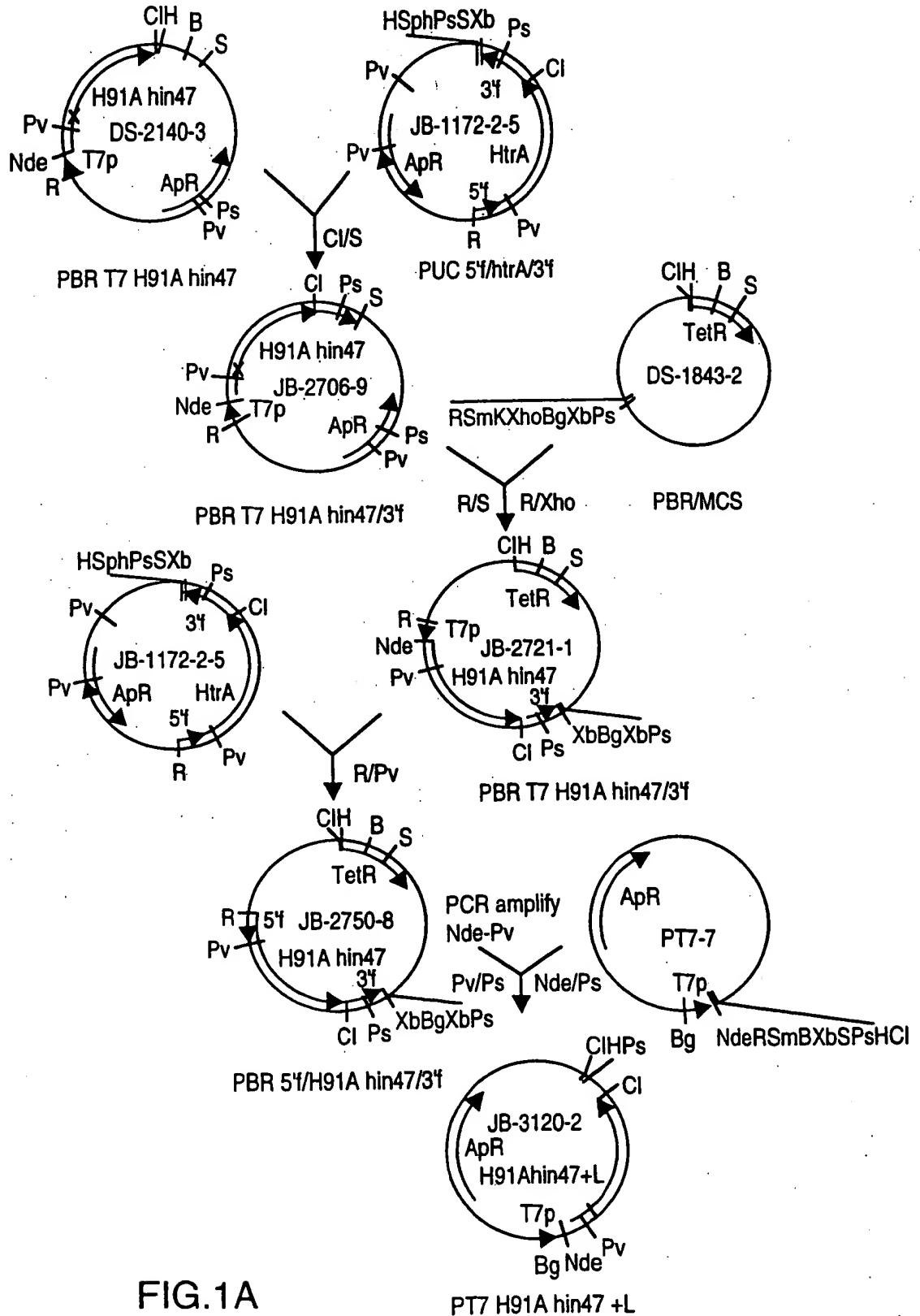


FIG.1A

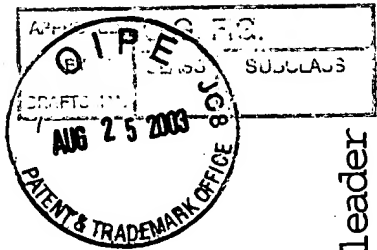


FIG.1B

Oligonucleotide primers to PCR amplify the Nde I-Pvu I fragment encoding the leader sequence of HtrA.

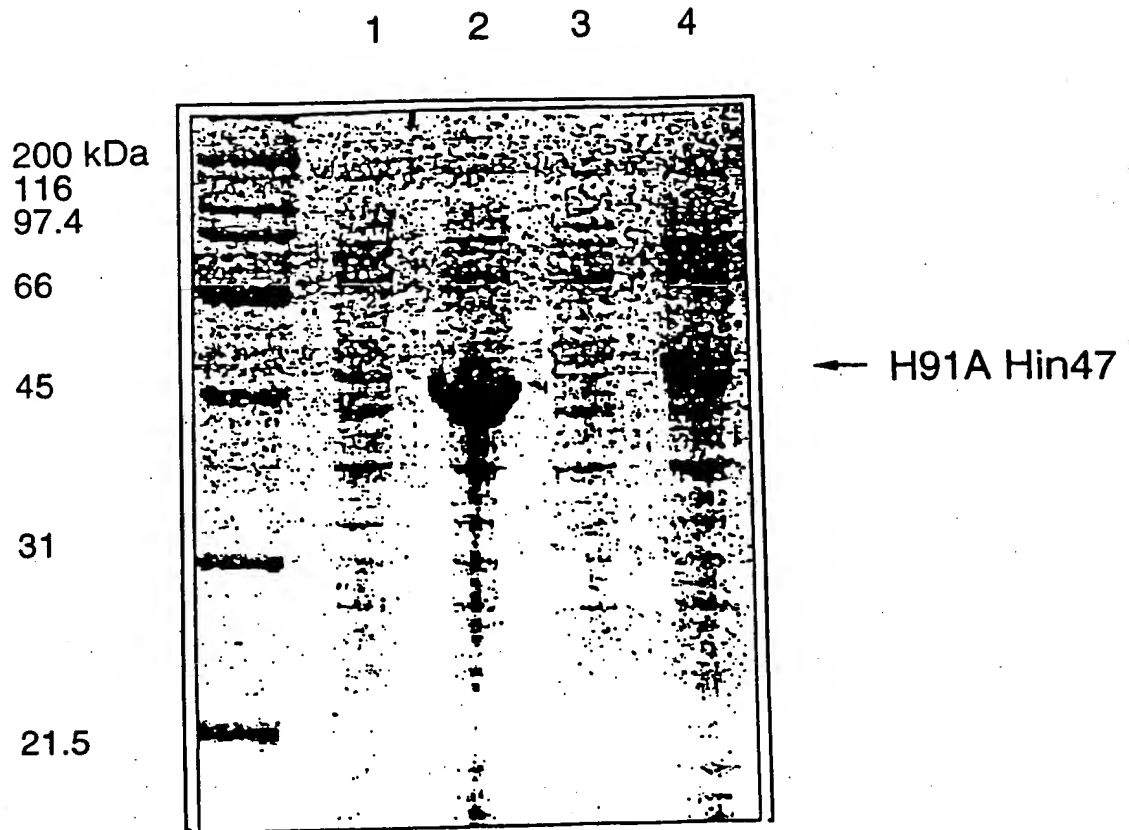
Nde I				
	M	K	K	T
5'	GGCGCATATC	AAAAAA	CACGTTT	TGTACTAAATAGT
			3'	6931.SL
				SEQ ID No.2
				SEQ ID No.1

Pvu I				
	F	K	F	F
3'	TTTAAATCTCTTTCG	CGATCG	TTTTCGCCGAACAA	
	AAATTTAAGAACAA	CCGCTAGC	AAAAACGGCTTGT	
		5'	6932.SL	
				SEQ ID No.5
				SEQ ID No.4
				SEQ ID No.3

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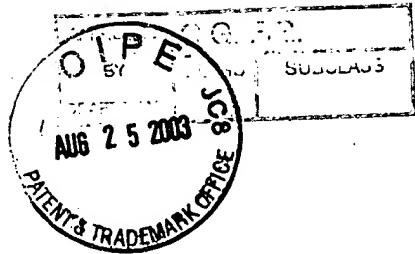
200 kDa  
116  
97.4  
66  
45  
31  
21.5

# Production of H91A Hin47 +/- leader



1. H91A Hin47 (- leader)  $t_0$
2. H91A Hin47 (- leader)  $t_4$
3. H91A Hin47 (+ leader)  $t_0$
4. H91A Hin47 (+ leader)  $t_4$

FIG.2



# Purification of H91A Hin47 (+ leader) from *E. coli*

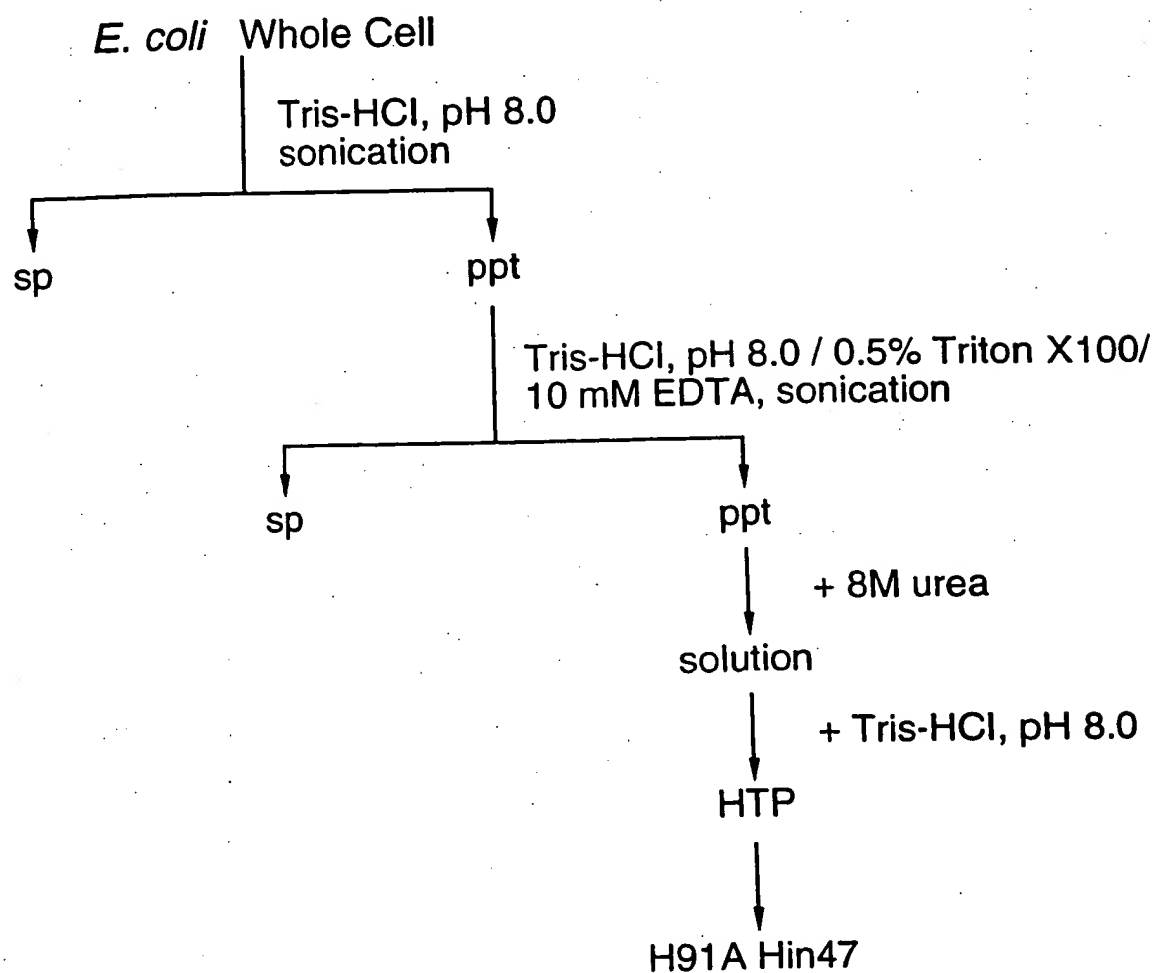
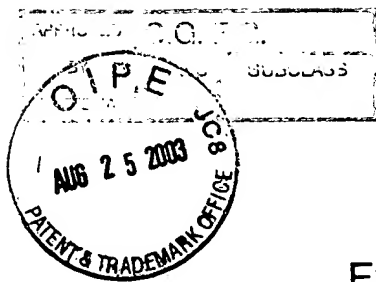
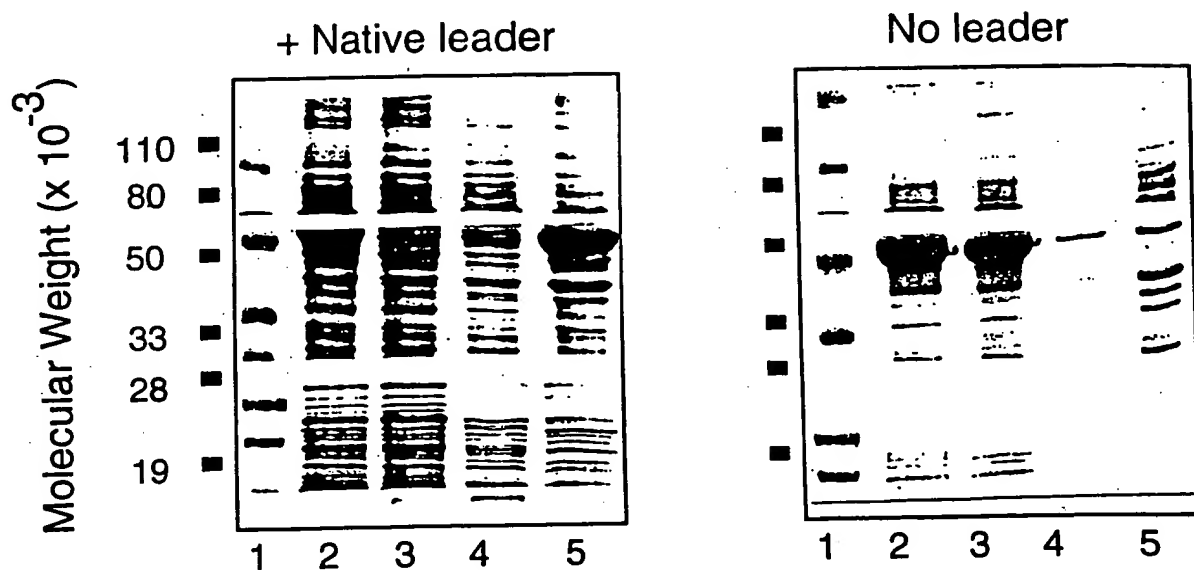


FIG.3

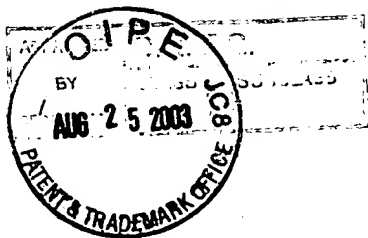


Extraction of H91A Hin47 +/- leader, produced in *E. coli*



1. Pre-stained markers
2. *E. coli* whole cells
3. Soluble proteins in 50mM Tris-HCl extraction
4. Soluble proteins in Tris/EDTA/Triton-100 extraction
5. Remaining pellets

FIG.4



Construction of DS-2342-2-2, a plasmid containing T7 H91A *hin47* and T7 V38 *hia*

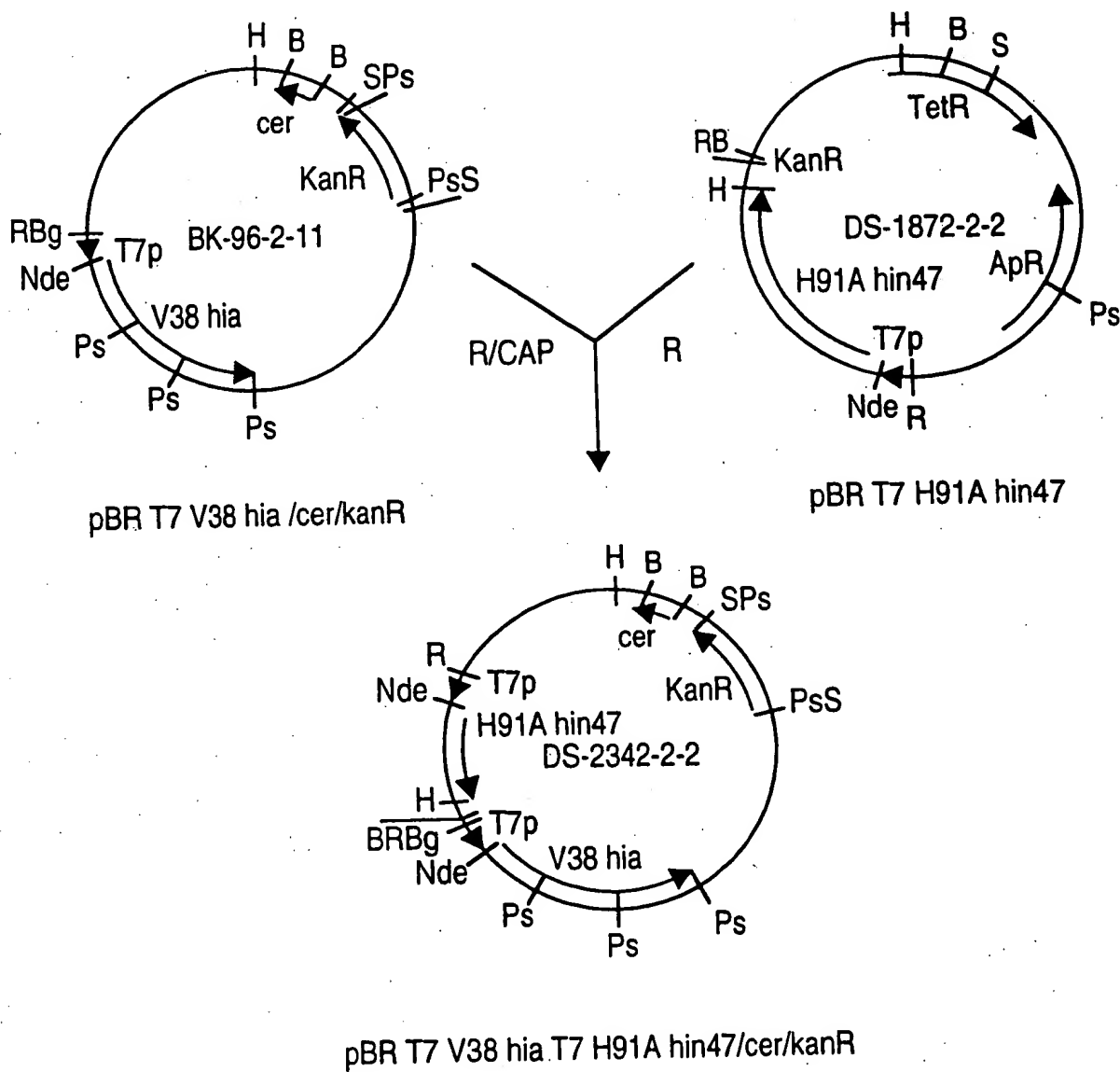
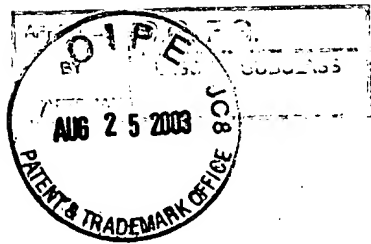
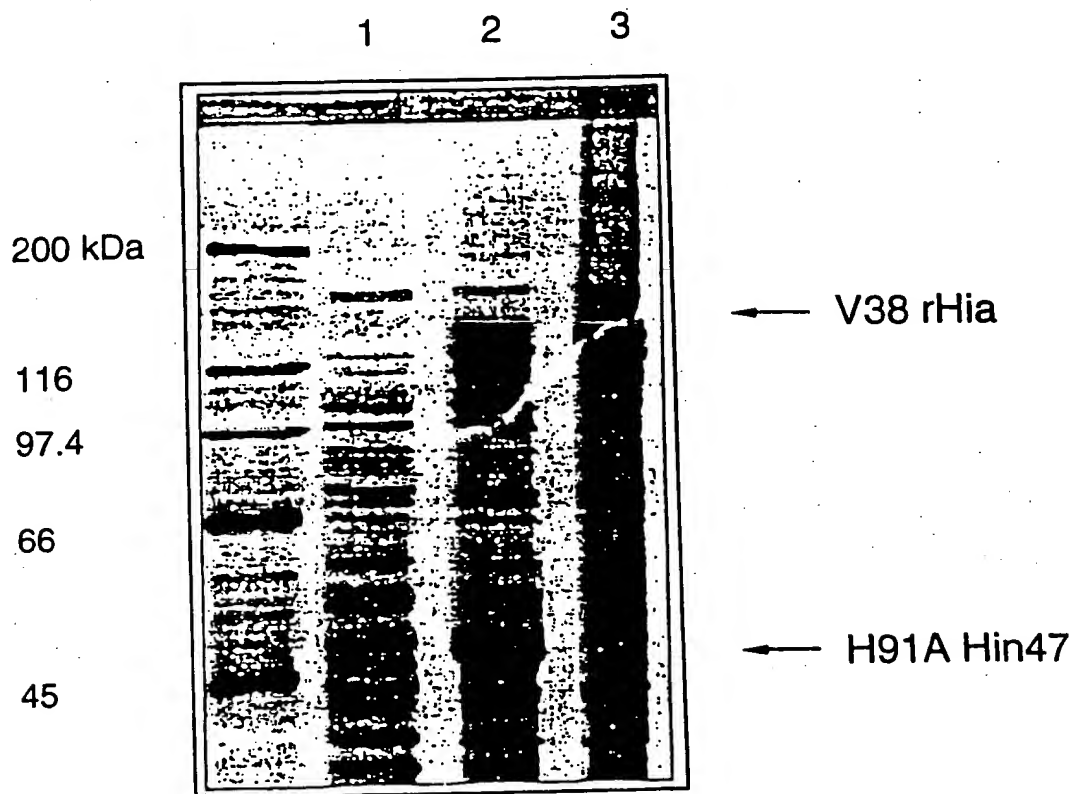


FIG.5

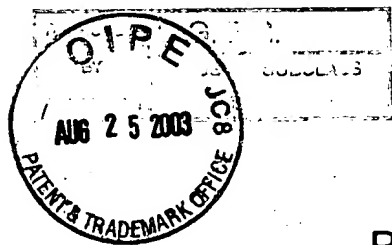


# Production of V38 rHia and H91A Hin47 when co-expressed



1. V38 rHia and H91A Hin47  $t_0$
2. V38 rHia and H91A Hin47  $t_4$
3. V38 rHia alone  $t_4$

FIG.6



## Purification of H91A Hin47 and V38 rHia from *E. coli*

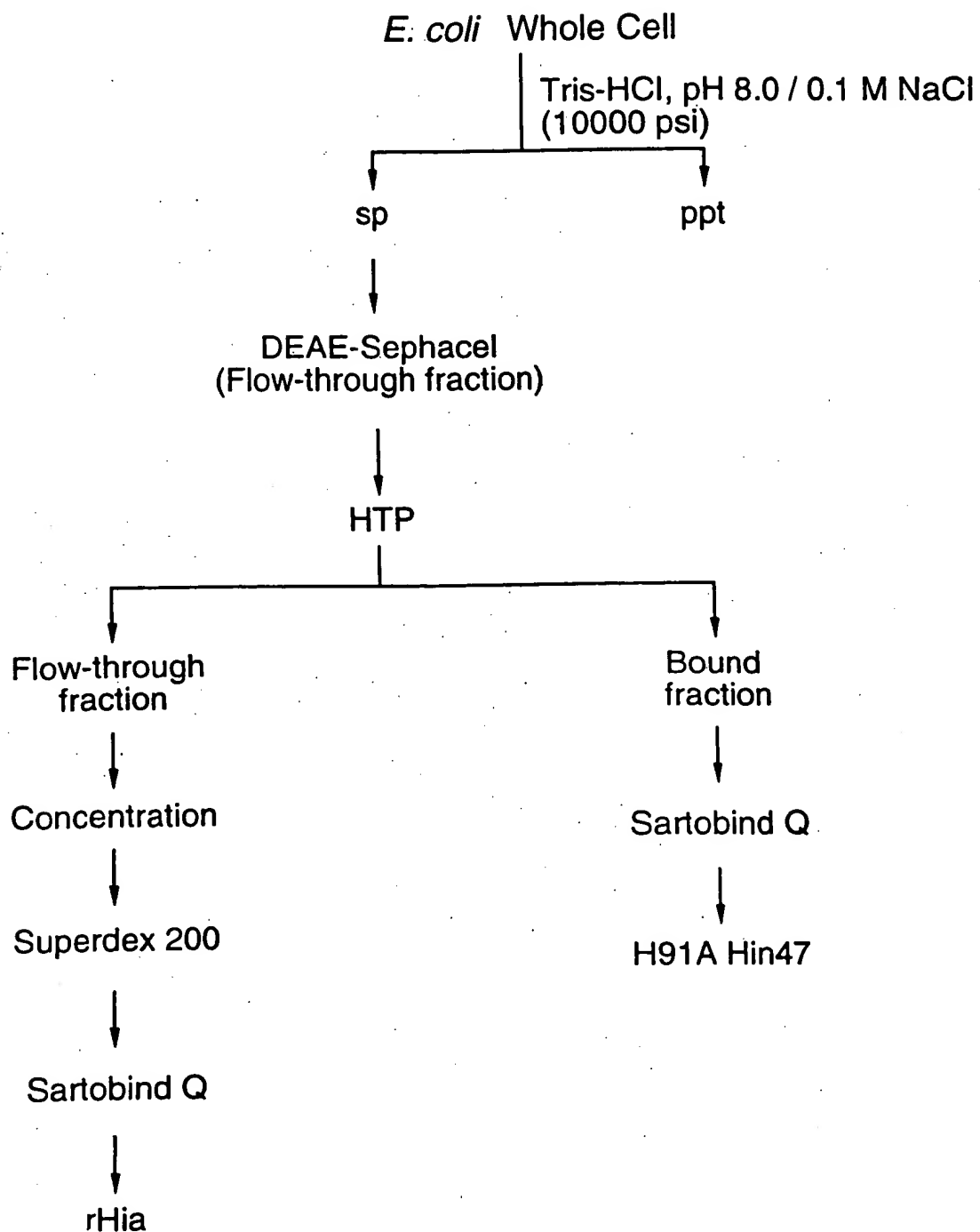
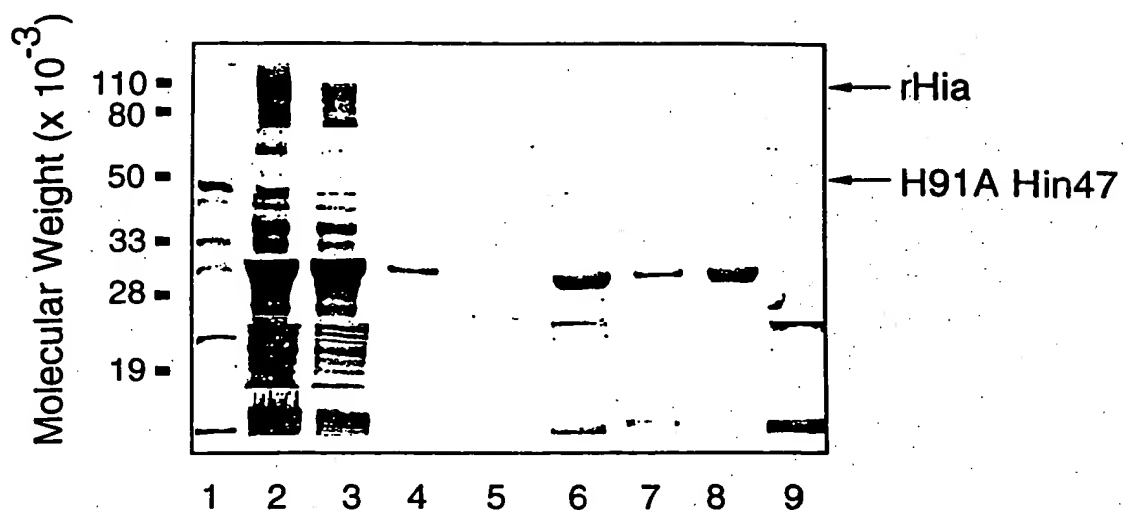


FIG.7



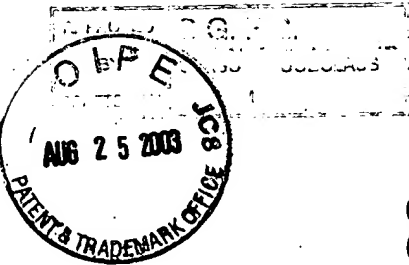


Purification of H91A Hin47 and V38 rHia from *E. coli*



- |  |                        |
|--|------------------------|
| 1. Prestained molecular weight markers | 6. DEAE flow-through   |
| 2. <i>E. coli</i> whole cell lysate    | 7. HTP flow-through    |
| 3. Tris/NaCl extract                   | 8. Purified H91A Hin47 |
| 4. Tris/ Triton X-100 / EDTA extract   | 9. Purified rHia       |
| 5. Tris/ OG extract                    |                        |

FIG.8



Construction of JB-3134-1-1, a plasmid containing T7 H91A hin47 (-leader) and T7 S44 hia

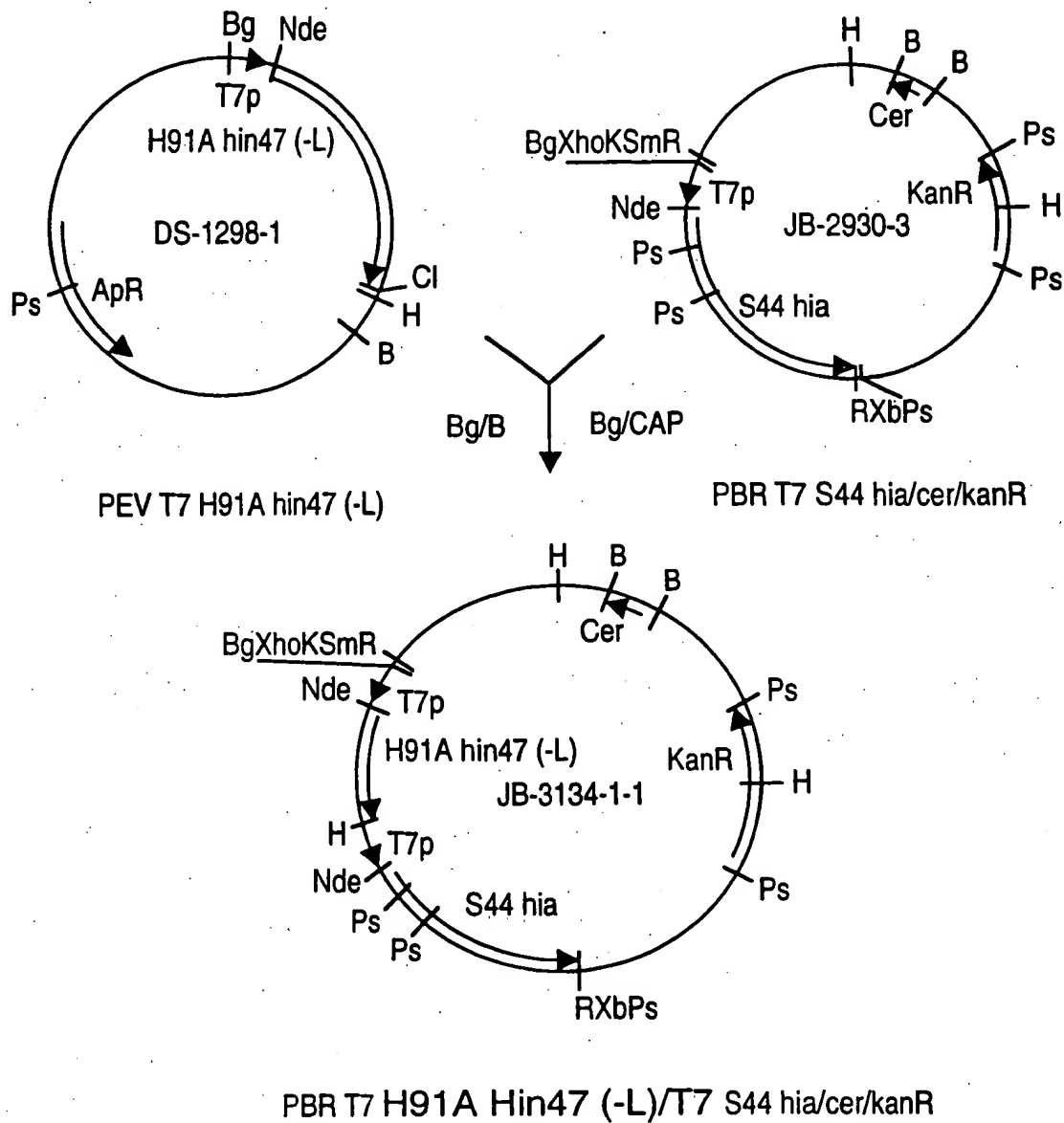
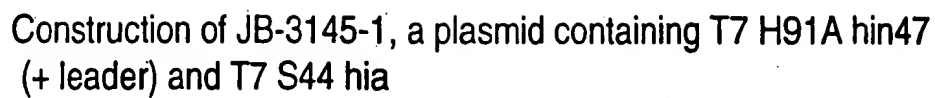
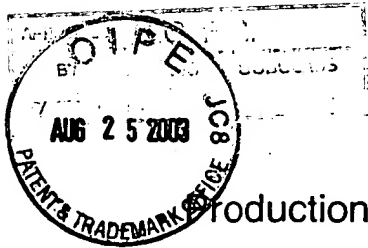
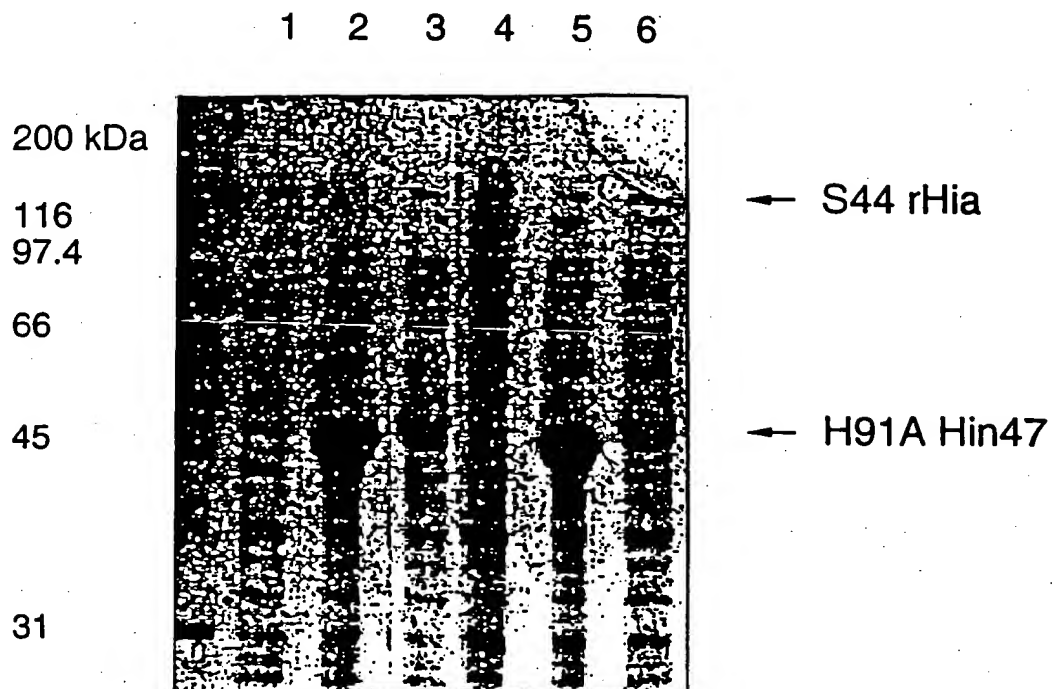


FIG.9





Production of S44 rHia and H91A Hin47 (+/-L), when co-expressed.



- |                               |                |
|-------------------------------|----------------|
| 1. H91A Hin47 (-L)            | t <sub>0</sub> |
| 2. H91A Hin47 (-L)            | t <sub>4</sub> |
| 3. H91A Hin47 (+L)            | t <sub>4</sub> |
| 4. S44 rHia                   | t <sub>4</sub> |
| 5. H91A Hin47 (-L) + S44 rHia | t <sub>4</sub> |
| 6. H91A Hin47 (+L) + S44 rHia | t <sub>4</sub> |

FIG.11



Construction of JB-3073R-1, a plasmid containing T7 psaA (+ leader) and T7 H91A hin47

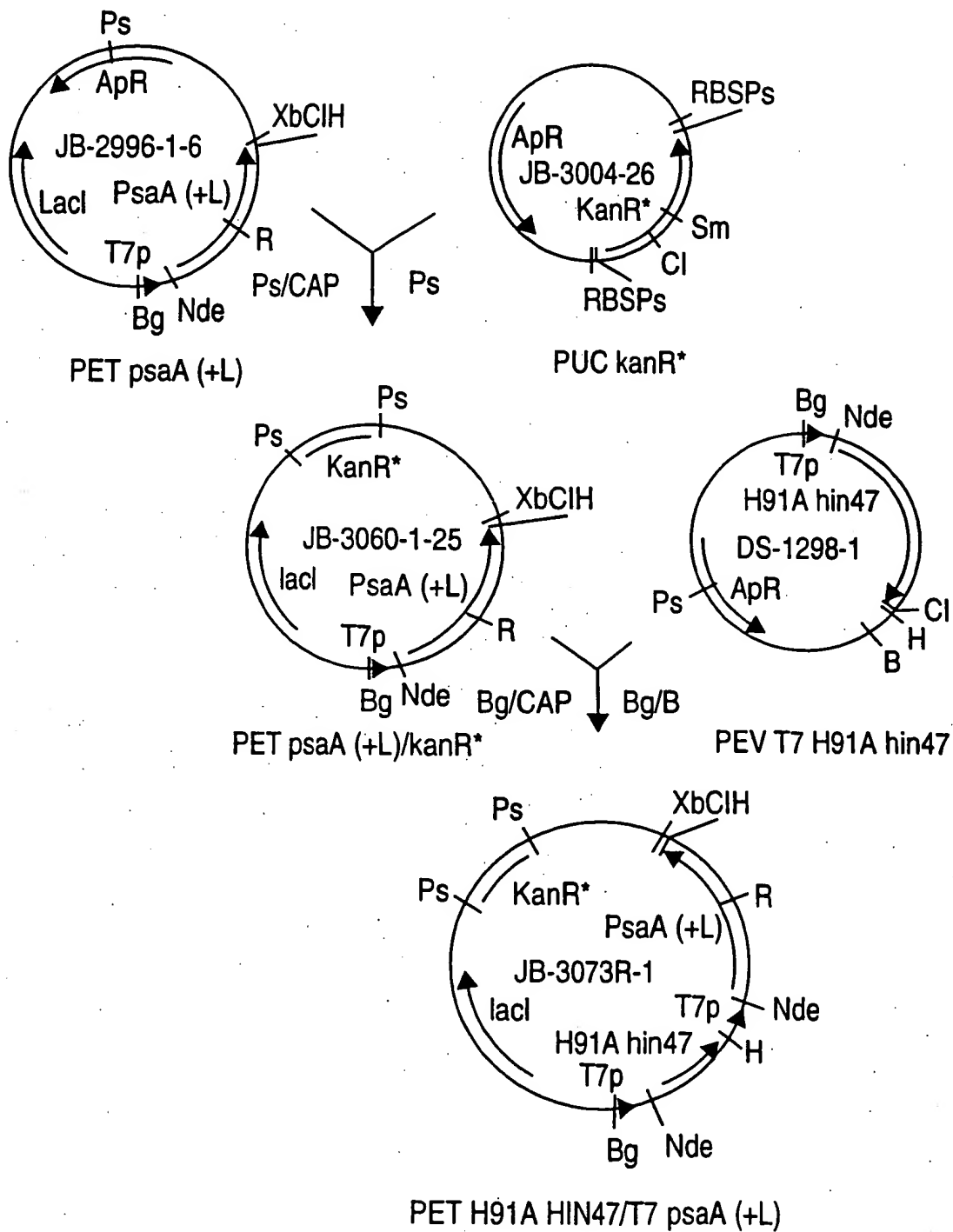
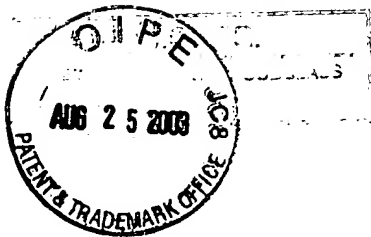


FIG.12A





Construction of JB-3090-1 and JB-3090-7, plasmids containing  
T7 psaA (-leader) and T7 H91A hin47

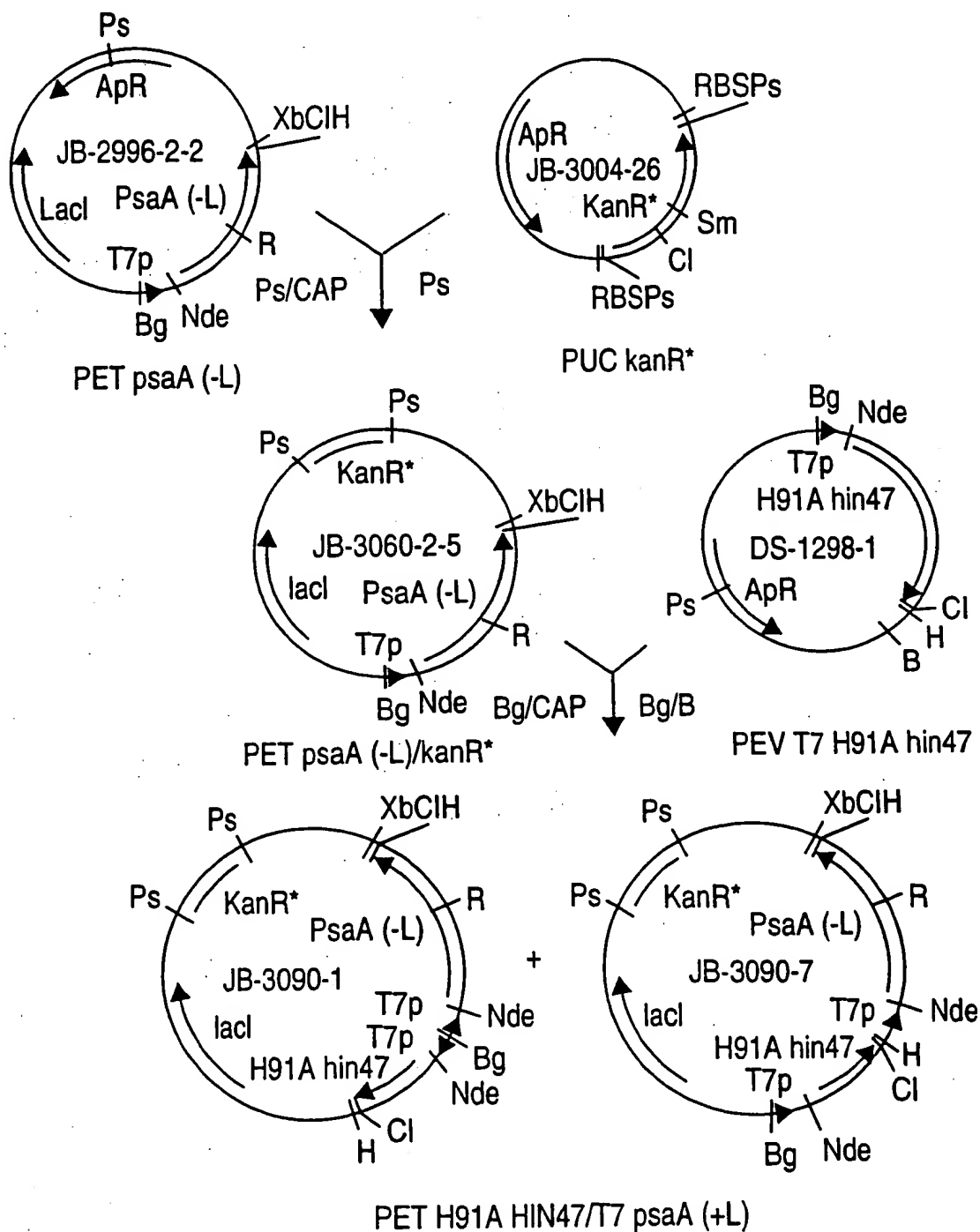
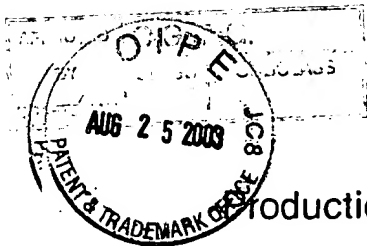


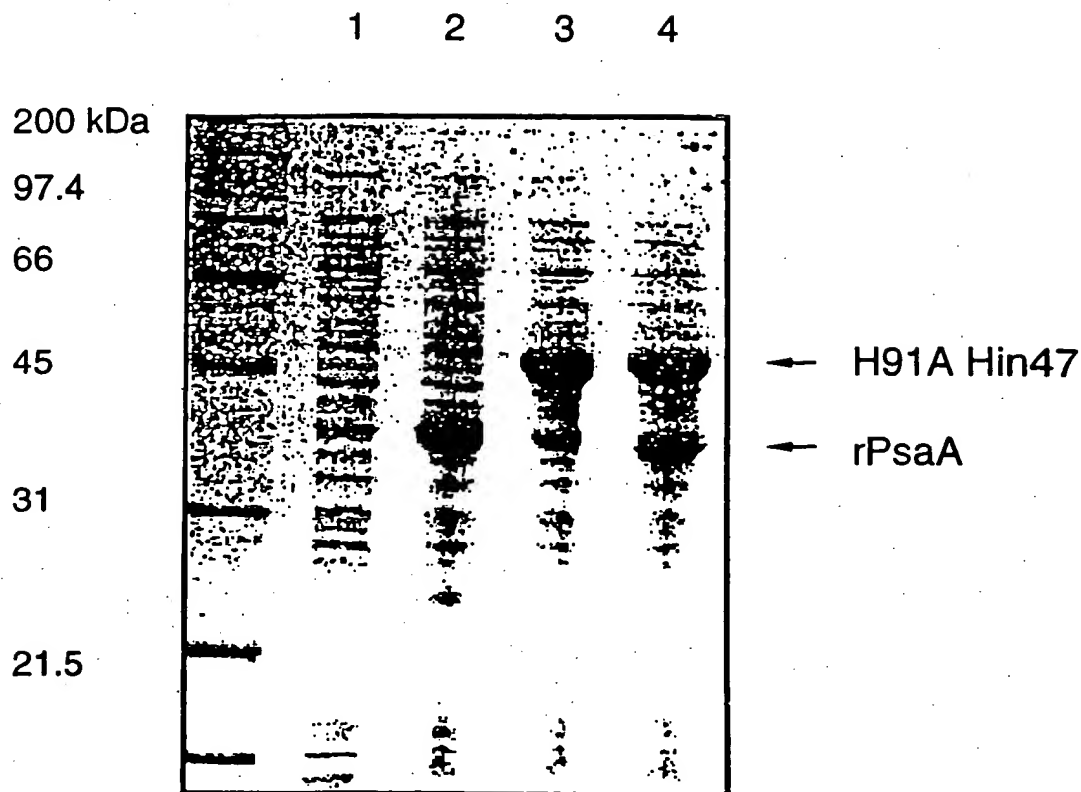
FIG.13A





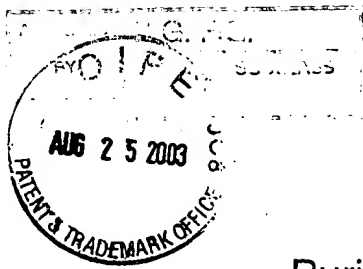


roduction of rPsaA +/- leader and H91A Hin47, when co-expressed.



1. H91A Hin47 and rPsaA (+ leader), < > orientation t<sub>0</sub>
2. H91A Hin47 and rPsaA (+ leader), < > orientation t<sub>4</sub>
3. H91A Hin47 and rPsaA (+ leader), > > orientation t<sub>4</sub>
4. H91A Hin47 and rPsaA (- leader), > > orientation t<sub>4</sub>

FIG.14



# Purification of H91A Hin47 and rPsaA from *E. coli*

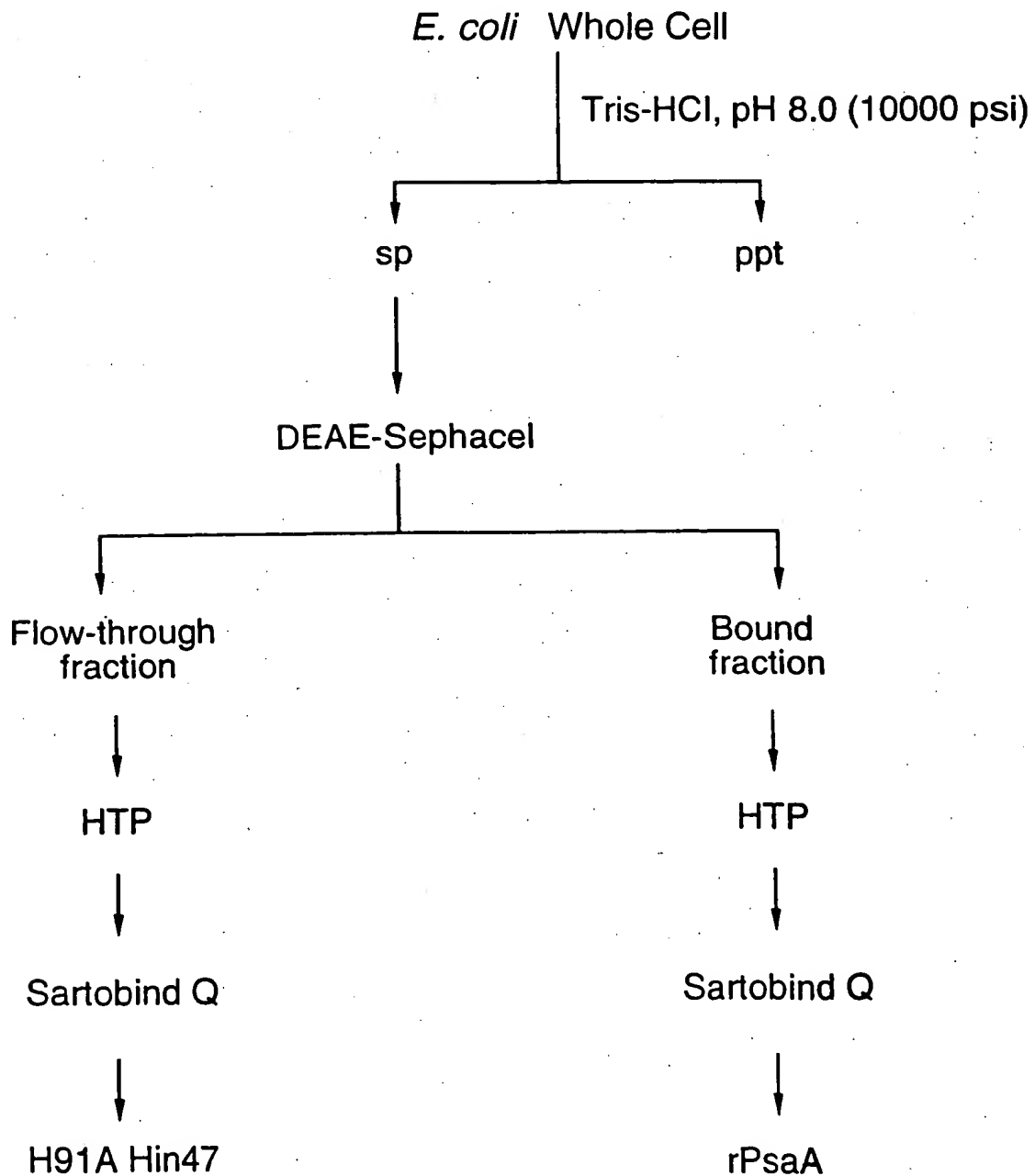
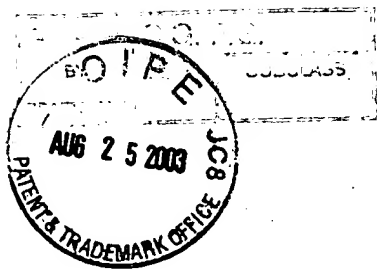
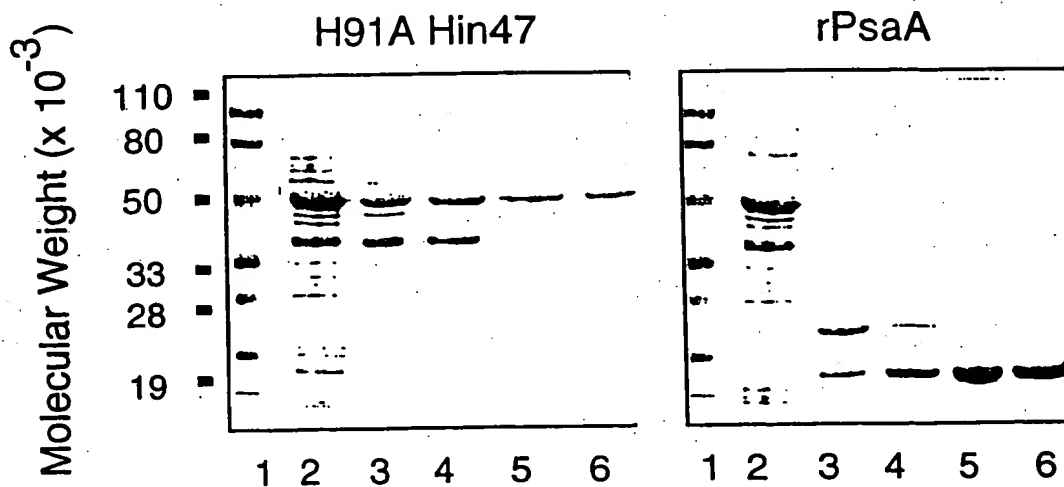


FIG.15



# Purification of H91A Hin47 and rPsaA from *E. coli*



1. Pre-stained molecular weight markers
2. *E. coli* whole cell lysate
3. Soluble proteins in 50mM Tris-HCl, pH 8.0 extraction
4. DEAE-Sephacel column
5. HTP column
6. Sartobind Q membrane

FIG.16

# Construction of a plasmid containing T7 hmwBC

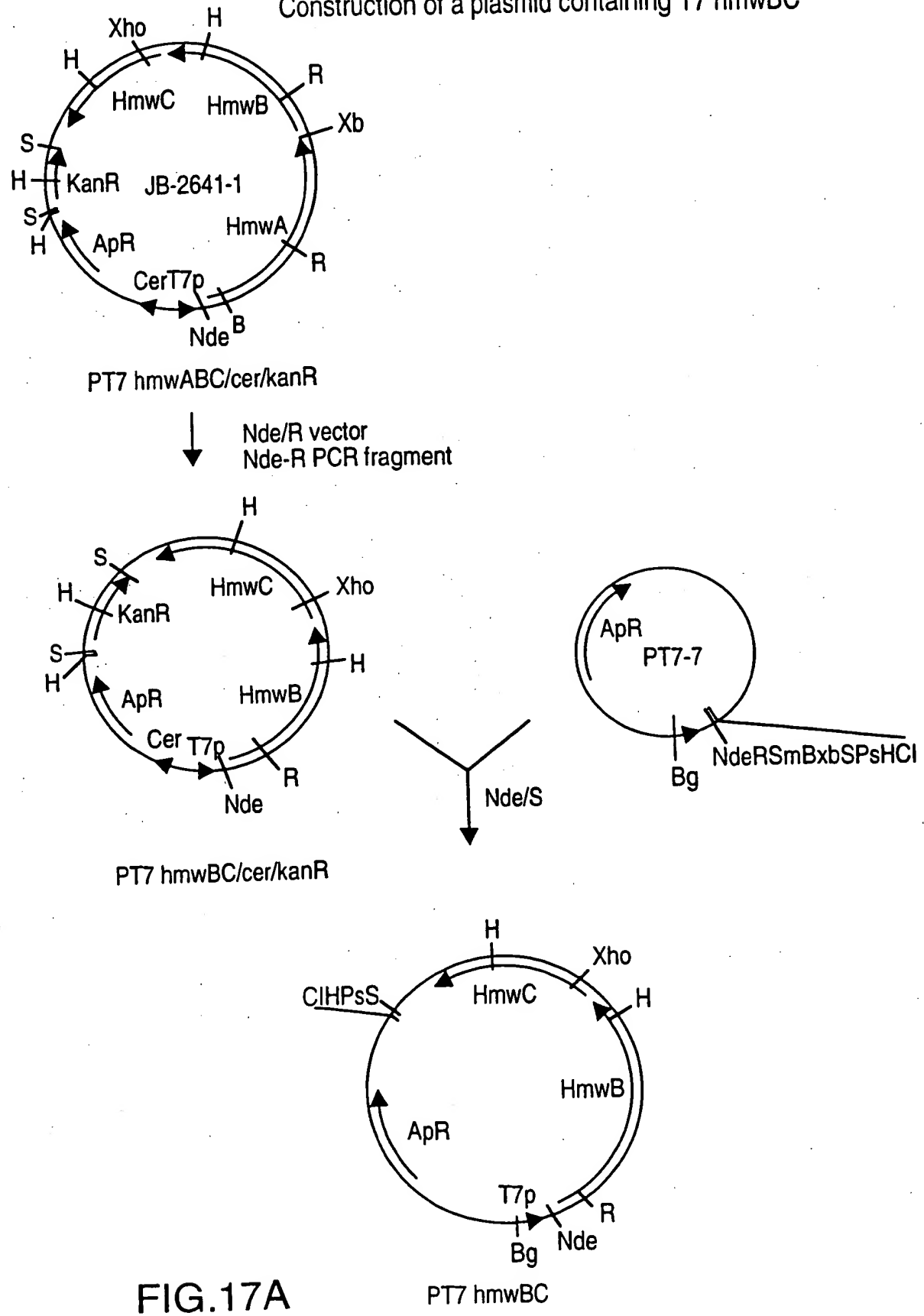


FIG.17A

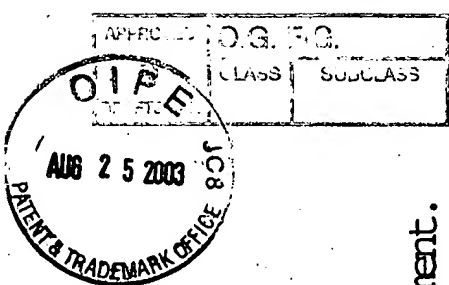


FIG.17B

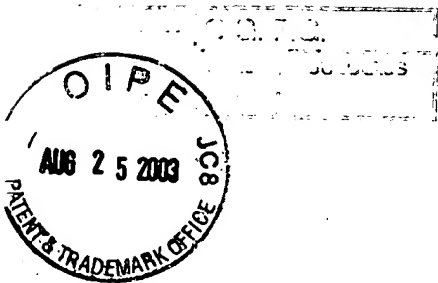
Oligonucleotide primers for PCR amplification of Nde I-EcoR I 5' *hmmB* fragment.

coding strand:

Nde I		
	M K N I K S R L K L	
5'	GGCGC <u>CATATG</u> <u>AAAAATATATAAA</u> ACCAGATTA AACTC	SEQ ID No:7
	3' 7072.SL	SEQ ID No:6

non-coding strand:

EcoR I		
	G R Q W F D L R E F N M A	
	GGTCGTCAGTCGGTTCGATTTCGGT <u>CAATTC</u> AATATGGCA	
3'	CCAGCAGTCACCAAGCTAAACGCACCTTAAGTTATACCGT	5' 5950.SL
		SEQ ID No:10
		SEQ ID No:9
		SEQ ID No:8



# Construction of plasmid containing T7 hmwB

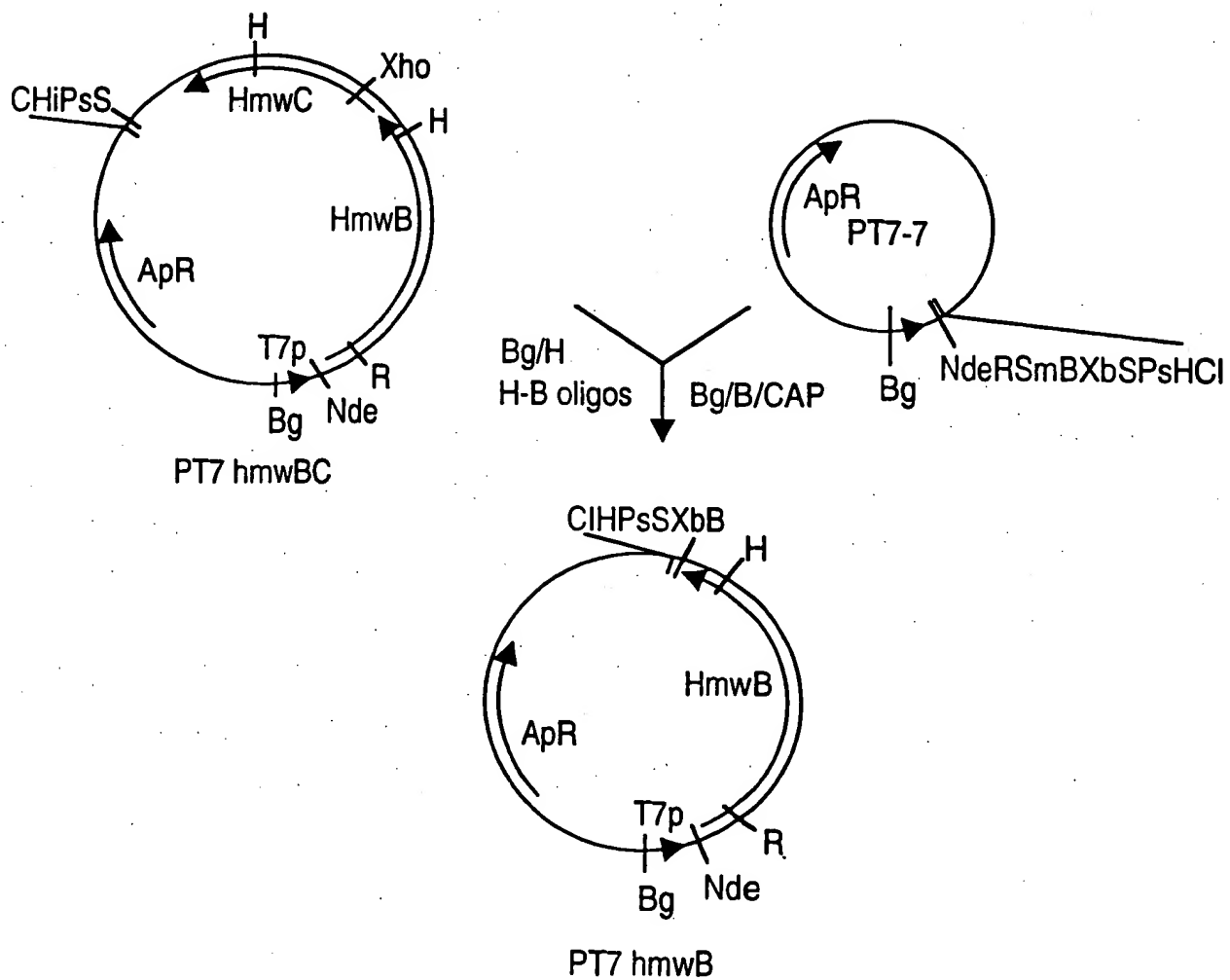


FIG.18A



FIG.18B

Oligonucleotides for construction of the Hind III-BamH I 3' *hmvB* fragment.

Hind III

S L D A F V A R R F A N A N S D N L N G N K K R T S S P T T...  
AGCTTAGATGCTTTTGTGTGCTGCTTTCGAAATGCCAATAGTACACATTTGAATGCCACAAAAA

... F W G R L T F S F \* \*  
...  
...CTTCTGGGGTAGATTAAACATTCAGTTTCTAATAG  
CGCACAAAGCTCACTACAAC...  
SEQ ID No:13  
7073.SL  
SEQ ID No:11  
7074.SL  
SEQ ID No:12

ATCTACGAAACAACGACGACGCGAAACGTTTACGGTTATCACTGTAAACTTACCGTTGTTTTCGGTGTTCGAG

...  
TCGATGTGTG...

...  
...GAAGACCCCATCTAATTGTAAAGTAAAGATTATCTAG  
...  
7076.SL  
7075.SL  
SEQ ID No:15  
SEQ ID No:14  
BamH I



# Construction of a plasmid containing T7 hmwC

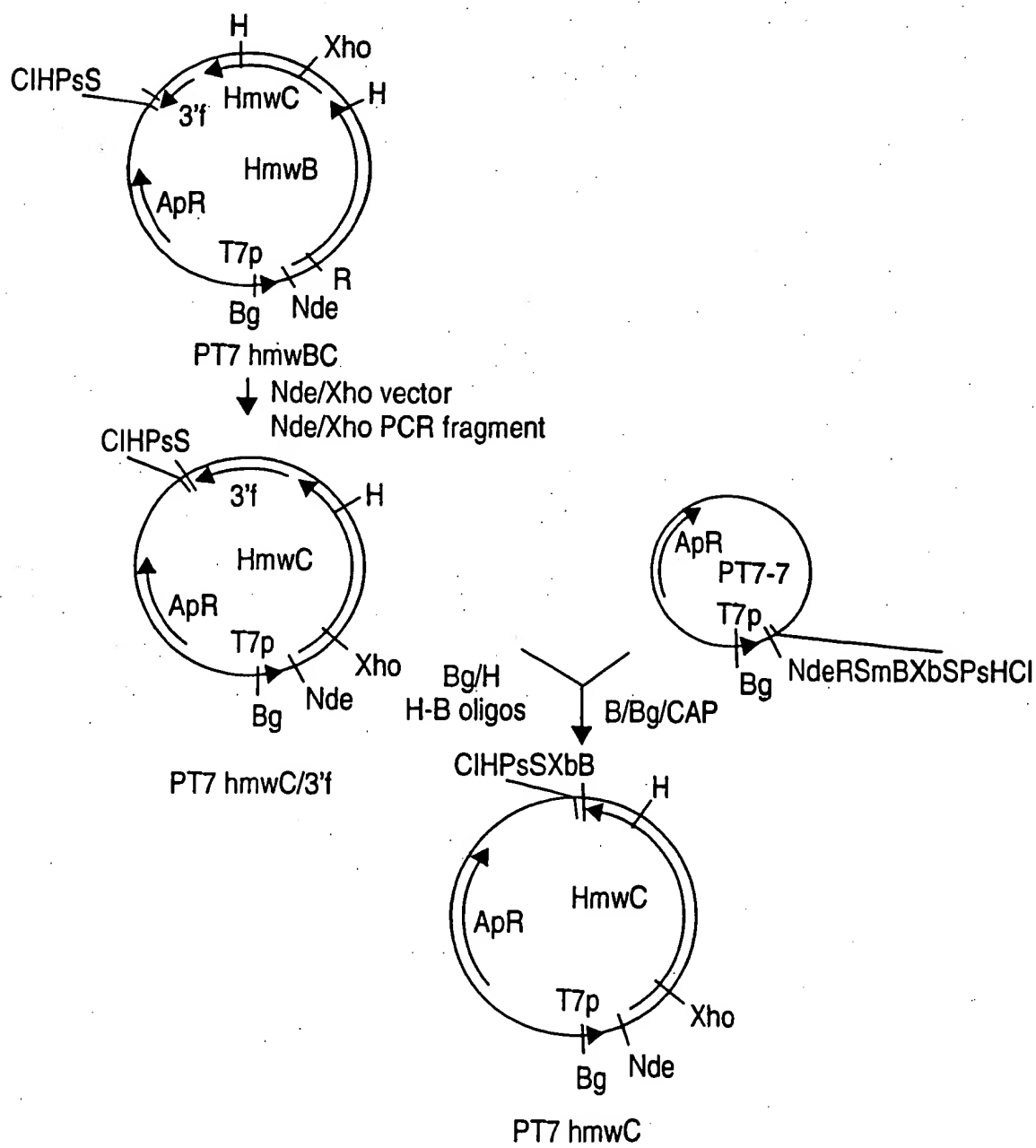


FIG.19A



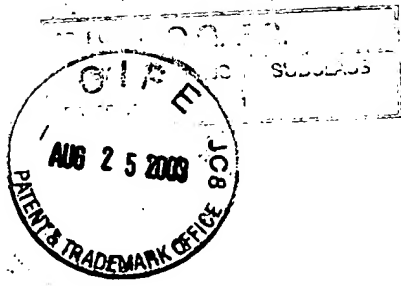


FIG.19B

Oligonucleotide primers for PCR amplification of Nde I-Xho I 5' hmwC fragment.

coding strand:

Nde I			
	M T K E N L Q S V P		SEQ ID No:17
5'	GGCCCATATGACAAAGAAANTTACAAAGIGTTCA	3' 7077.SL	SEQ ID No:16

non-coding strand:

Xho I			
	S T S M I A A R E K F Y		SEQ ID No:20
	TCAACTTCAATGATGCTCTCGACAAAATTTCTAT		SEQ ID No:19
3'	AGTTGAAGTTACTAACGACGAGCTCTTTTAAAGATA	5' 7078.SL	SEQ ID No:18

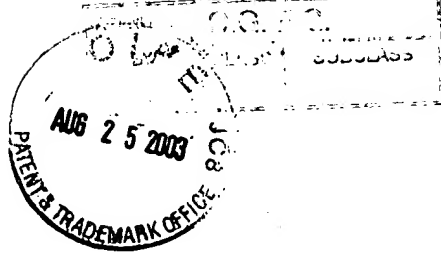


FIG.19C

Oligonucleotides for construction of Hind III-BamH I 3' *lmmC* fragment..

Hind III

L F T G D P R P L G K I L L K K T N E W...

AGCTTTTACAGCGAACCTGTCCTGTCATGGGCAAAATA

... K R K H L S K K \* \* \* \*  
CTGCTTAAGAAACAAATGAATG...

SEQ ID No:23

7079.SL

SEQ ID No:21

7080.SL

SEQ ID No:22

... GAAGCGAAGCACTTCAGTAAAAAATAATAG

AAATGTCGCTCGGAGCAGGTAAACCGTTTATGACGAATTC

TTTGTGTTTACTTAC...

7082.SL SEQ ID No:25

7081.SL SEQ ID No:24

...CTTCGCTTCGTCGACCTATTTTATTATTCCTAG

BamH I